

Amendments to the Specification

Please add the following paragraph:

RELATED APPLICATIONS

This application is the U.S. National Stage of International Application No. PCT/JP2003/013768, filed October 28, 2003, published in Japanese, and claims priority under 35 U.S.C. § 119 or 365 to Japan Application No. 2002-313534, filed October 28, 2002.

Please replace the paragraph at page 4, line 26 - page 5, line 7 with the following amended paragraph:

The present inventors conducted intensive studies on structures wherein the timing of contact of fluid substances placed in a number of cavities can be controlled without depending on a mechanical structure. Thus, they discovered that the transfer of fluid substances in a number of cavities isolated by an intervening intermediate cavity can be controlled by introducing a medium to the intervening intermediate cavity. The present inventors also discovered that using this technique enables the control of not only fluid substances, but also of substances comprised in the fluid substances, completing the present invention. The present invention relates to methods for controlling fluid substances and/or substances comprised in fluid substances, and devices for realizing such control methods, as described below. The present invention also relates to methods of electrophoretic analysis using such methods, and devices for such methods.

Please replace the paragraph at page 5, lines 8-20, with the following amended paragraph:

[1] A method for controlling the transfer of a fluid substance and/or a substance comprised in a fluid substance, from the first cavity to the second cavity, comprising the steps of:

a) introducing into the first cavity, a fluid substance whose transfer is to be controlled and/or a substance whose transfer is to be controlled comprised in the fluid substance, and holding the fluid substance and/or the substance comprised in the fluid substance in the first cavity, wherein the first cavity is connected to the second cavity by an intervening intermediate cavity, and the intervening intermediate cavity is provided with a separation medium which prevents transfer of the fluid substance and/or the substance comprised in the fluid substance into the intervening intermediate cavity;

b) replacing the separation medium in the intervening intermediate cavity with a connection medium that allows transfer of the fluid substance and/or the substance comprised in the fluid substance into the intervening intermediate cavity; and

c) transferring the fluid substance and/or the substance comprised in the fluid substance from the first cavity to the second cavity via the intervening intermediate cavity.

Please replace the paragraph at page 6, lines 1-3 with the following amended paragraph:

[4] The method of [1], wherein the first cavity, the second cavity, and the intervening intermediate cavity comprise a groove or tube configuration; the fluid substance is a liquid; the separation medium is a gas; and the connection medium is liquid.

Please replace the paragraph at page 6, lines 6-16 with the following amended paragraph:

[6] A device for controlling the transfer of a fluid substance and/or a substance comprised in a fluid substance, comprising:

a) a first cavity for holding the fluid substance;
b) a second cavity for holding the fluid substance; and
c) an intervening intermediate cavity for connecting the first cavity and the second cavity, for holding a separation medium which prevents transfer of the fluid substance and/or the substance comprised in the fluid substance from the first cavity; wherein the separation medium can be replaced with a connection medium, and the introduction of the connection medium to the intervening intermediate cavity enables transfer of the fluid substance and/or the substance comprised in the fluid substance retained in the first cavity to the intervening intermediate cavity and the second cavity.

Please replace the paragraph at page 6, line 25 - page 7, line 6 with the following amended paragraph:

[8] A two-dimensional electrophoretic device comprising;
a) a first cavity for holding an electrophoretic medium;
b) a second cavity for holding an electrophoretic medium; and
c) an intervening intermediate cavity for connecting the first cavity and the second cavity, for holding a separation medium which prevents transfer of the substance to be electrophoresed from the first electrophoretic medium to the second electrophoretic medium;

wherein the separation medium can be replaced with a connection medium, and the introduction of the connection medium to the intervening intermediate cavity enables transfer of the substance to be electrophoresed in the electrophoretic medium retained in the first cavity to the intervening intermediate cavity and the second cavity.

Please replace the paragraph at page 7, lines 9-12 with the following amended paragraph:

[10] A method for conducting two-dimensional electrophoresis comprising the steps of:

- a) conducting electrophoresis in the first cavity of the electrophoretic device of [8];
- b) introducing a connection medium to an intervening intermediate cavity after step a); and
- c) conducting electrophoresis of a substance to be electrophoresed in the second cavity.

Please replace the paragraph at page 7, lines 14-27 with the following amended paragraph:

The present invention relates to a method for controlling the transfer from a first cavity to a second cavity of a fluid substance and/or a substance comprised in a fluid substance, comprising the steps of:

- a) introducing into the first cavity a fluid substance whose transfer is to be controlled, and/or a substance whose transfer is to be controlled comprised in the fluid substance, and holding the fluid substance and/or the substance comprised in the fluid substance in the first cavity, wherein the first cavity is connected to the second cavity by an intervening intermediate cavity, and the intervening intermediate cavity is provided with a separation medium which prevents transfer of the fluid substance and/or the substance comprised in the fluid substance into the intervening intermediate cavity;
- b) replacing the separation medium in the intervening intermediate cavity with a connection medium that allows transfer of the fluid substance and/or the substance comprised in the fluid substance into the intervening intermediate cavity; and
- c) transferring the fluid substance and/or the substance included in the fluid substance from the first cavity to the second cavity via the intervening intermediate cavity.

Please replace the paragraph at page 8, line 26 - page 9, line 2 with the following amended paragraph:

When the support is constructed by connecting a number of components, the components are preferably connected such that each component can not be easily detached. Specifically, structures preferred in the present invention include supports connected by means such as adhesion, fusion, screwing, and fitting. For example, a number of grooves may be formed on the surface of one planar support, and each of the grooves may be used as a first and or a second cavity.

Please replace the paragraphs at page 10, lines 4 - page 12, line 1 with the following amended paragraphs:

In the present invention, an intervening intermediate cavity is provided between the first and the second cavities, such that the intervening intermediate cavity connects the two cavities. Transfer of fluid substances and/or substances comprised in the fluid substances from the first cavity to the second cavity may be restricted by placing a separation medium in the intervening intermediate cavity. A separation medium of the present invention is a medium comprising the function of preventing transfer to the intervening intermediate cavity of either one or both of the fluid substances, and the substances comprised in the fluid substances, which are accommodated in the first cavity. The term “materials substances to be moved transferred” is hereinafter used to represent one or both of the fluid substance, and substances comprised in the fluid substance, accommodated in a first cavity of the present invention.

Gas or liquid may be used as a separation medium. For example, when the first cavity holds aqueous media, transfer of the aqueous media to an intervening intermediate cavity can be restricted by filling the intervening intermediate cavity with air. One example of a basic structure for carrying out the control methods of the present invention can be a structure whereby the first cavity comprises one groove, the second cavity comprises a second groove that intersects the first cavity, and the intervening intermediate cavity comprises a third groove that intersects the second cavity groove. The third groove is located near the intersection of the first and second cavities. In this structure, the liquid accommodated in the first cavity can not be transferred to the second cavity without passing through the intervening intermediate cavity (third groove). When air is present in the intervening cavity, the surface

tension of the aqueous medium in the first cavity (the first groove) prevents this aqueous medium from entering the intervening intermediate cavity. As a result, the transfer of the aqueous medium in the first cavity is controlled.

The connection medium of the present invention is a medium which, upon contact with the "material substances to be moved transferred", enables the transfer of this substance to the second cavity. When the material substances to be moved transferred is a fluid substance, liquid miscible with the fluid substance can be used as the connection medium. The fluid substance is transferred to the second cavity through the connection medium in the intervening intermediate cavity, by physical energy such as diffusion into the miscible liquid, electroosmotic flow, centrifugal force, or the like. When the material substance to be moved transferred is a substance comprised in the fluid substance, it can be transferred to the second cavity through the connection medium in the intervening intermediate cavity by diffusion or electricity.

When the material substance to be moved transferred, which is accommodated in the first cavity, is a liquid, the separation medium may be a liquid immiscible with this material. By introducing the intervening intermediate cavity with a miscible liquid connection medium instead of an immiscible liquid, the material substance to be moved transferred can be guided into the intervening intermediate cavity.

The number of intervening intermediate cavities is not limited in the present invention. The present invention controls the transfer of a material substance to be moved transferred in the first cavity, by selecting which separation media and connection media is placed in the intervening intermediate cavity. Accordingly, by increasing the number of intervening intermediate cavities, the number of steps controlling the material substance to be moved transferred can be increased. More specifically, one intervening intermediate cavity can control the transfer of the material substance to be moved transferred at least once. When a number of second cavities are connected to the first cavity, transfer of the material substance to be moved transferred to the multiple second cavities can be started at once, by providing one intervening intermediate cavity.

Fig. 1 shows an example of such a structure. As shown in Fig. 1, provision of one two intervening intermediate cavity cavities intersecting a number of second cavities enables simultaneous control of the transfer of a material substance to be moved transferred to a number of second cavities. Alternatively, a number of intervening intermediate cavities can

be provided for a number of second cavities, to independently control each intervening intermediate cavity. For example, all second cavities may be separately provided with an intervening intermediate cavity, to thereby independently control transfer of the material substance to be moved transferred to all second cavities. This independent provision of intervening intermediate cavities prevents mixing of the material substance to be moved transferred through the intervening intermediate cavity.

The second cavities of the present invention may or may not comprise a fluid substance prior to receiving the material substance to be moved transferred. When a fluid substance is present in the second cavity, it is desirable to restrict the transfer to the first cavity of fluid substances and/or substances comprised in the fluid substances in the second cavity, through the separation medium.

Please replace the paragraph at page 13, lines 12-26 with the following amended paragraph:

The methods for controlling substance transfer of the present invention may be carried out by a device with the structure below. In other words, the present invention relates to devices for controlling the transfer of fluid substances and/or substances comprised in the fluid substances, comprising the components described below. Please note the specific structures of the first, second, and intervening intermediate cavities constituting the devices of the present invention are as described above.

- a) a first cavity for holding a fluid substance;
- b) a second cavity for holding a fluid substance; and
- c) an intervening intermediate cavity for connecting the first and second cavity, and for holding a separation medium which prevents the inflow of fluid substances and/or substances comprised in the fluid substances from the first cavity;
wherein the separation medium can be replaced with a connection medium, and the introduction of the connection medium to the intervening intermediate enables transfer of fluid substances and/or substances comprised in the fluid substances in the first cavity to the intervening intermediate cavity and the second cavity.

Please replace the paragraph at page 16, line 22 - page 17, line 4 with the following amended paragraph:

Accordingly, the present invention provides two-dimensional electrophoretic devices comprising:

- a) a first cavity for holding an electrophoretic medium;
- b) a second cavity for holding an electrophoretic medium; and
- c) an intervening intermediate cavity for connecting the first cavity and the second cavity, and for holding a separation medium which prevents transfer of the substance to be electrophoresed from the first electrophoretic medium to the second electrophoretic medium; wherein the separation medium can be replaced with a connection medium, and introduction of the connection medium to the intervening intermediate cavity enables transfer of the substance to be electrophoresed, which is in the electrophoretic medium retained in the first cavity, to the intervening intermediate cavity and the second cavity.

Please replace the paragraph at page 17, lines 18-25 with the following amended paragraph:

Second dimensional separation in the present invention is conducted in the electrophoretic medium in the second cavity, irrespective of the type of drive principle employed. The second cavity is connected to the first cavity via an intervening intermediate cavity. The first and second cavities can be realized, for example, by a structure comprising at least two grooves that branch on a flat plane. In the present invention, a branched structure comprises grooves in the form of a T, Y, or cross, or a structure with a larger number of intersecting grooves. In addition, the structure may comprise a number of second cavities branching from a single first cavity, or a small number of second cavities branching from a number of first cavities.

Please replace the paragraphs at page 18, line 25 - page 19, line 12 with the following amended paragraphs:

The intervening intermediate cavity can separate the electrophoretic medium in the first cavity from the electrophoretic medium in the second cavity, as well as connect the first cavity with the second cavity. The intervening intermediate cavity can be realized, for example, by a third groove structure intersecting the second cavity branching from the groove that constitutes the first cavity, at a position beyond the branching point of the first and

second cavities. For example, assuming that the second grooves constituting the second cavity are placed perpendicular to the first cavity (first groove), a third groove placed along the first groove and intersecting with the second grooves may be provided as the intervening intermediate cavity. The third groove and the first groove do not need to be parallel, as long as they are side by side.

After completing electrophoresis in the first cavity, a connection medium is introduced to the intervening intermediate cavity. The substance separated in the electrophoretic medium in the first cavity (the substance to be electrophoresed) then moves in to the connection medium. The connection medium contacts the electrophoretic medium in the second cavity. Thus, the substance to be electrophoresed can now move into the electrophoretic medium in the second cavity. Electrophoresis of the substance in the second cavity can begin when voltage is applied under these conditions in the axial direction of the second cavity.

Please replace the paragraphs at page 21, lines 23 - page 23, line 5 with the following amended paragraphs:

In the present invention, an intervening intermediate cavity is provided between the first and second cavities. For example, as described above, when a third cavity intersecting with a second cavity is placed somewhere between the intersection of the first and second cavities and the second cavity, an intervening intermediate cavity with grooves in the relation described above can be achieved. That is, a third groove serving as the intervening intermediate cavity can be placed to extend along the second cavity and intersect with the second cavity(s).

The distance between the grooves serving as the first and intervening intermediate cavities is arbitrary. When this distance is great, a larger amount of protein in the first cavity may diffuse to the intervening intermediate cavity during isoelectric focusing. Therefore, the distance between these cavities is preferably short. Specifically, the intervening intermediate cavity may be placed at a distance of 10% to 200% of the width of the groove constituting the first cavity.

The number of the grooves constituting the intervening intermediate cavity is not particularly limited. For example, a pair of grooves may be placed on both sides of a first cavity as the intervening intermediate cavity. When such grooves placed on both sides of the

first cavity are connected at a position that does not intersect with the first or second cavity, the intervening intermediate cavities will constitute one U-shaped groove. On the other hand, when discontinuous grooves are used as the intervening intermediate cavities, intervening intermediate cavities formed from a number of grooves may be realized.

Separation medium, which prevents the transfer of substance to be electrophoresed, is placed in the intervening intermediate cavity. Exemplary separation media comprise gas such as air. Isoelectric focusing is conducted in the first cavity, with separation medium in the intervening intermediate cavity. During isoelectric focusing, the separation medium prevents diffusion of the substance to be electrophoresed into the denatured gel in the second cavity.

After the completion of isoelectric focusing, connection medium is introduced to the intervening intermediate cavity. A buffer may be used as the connection medium, and specific examples include Tris buffer, phosphate (sodium or potassium) buffer, glycine buffer, carbonate buffer, borate buffer, the Good's buffer series, citrate buffer, phthalate buffer, formate buffer, acetate buffer, succinate buffer, cacodylate buffer, imidazole buffer, and pyrophosphate buffer.

Introduction of a buffer enables diffusion of the material substance to be moved transferred from the first cavity to the intervening intermediate cavity. In this state, application of voltage to the second cavity starts electrophoresis in the second cavity, which is equipped with a denatured gel. More specifically, the substance to be electrophoresed, which was separated in the isoelectric focusing medium, will transfer to the nearest second cavity of each band. Two-dimensional electrophoresis separation methods that use two-dimensional electrophoretic separation devices of the present invention are thereby implemented.

The present invention provides methods for conducting two-dimensional electrophoresis comprising the steps of:

- a) conducting electrophoresis in a first cavity of an electrophoretic device as described above;
- b) introducing a connection medium to an intervening intermediate cavity after step a); and
- c) conducting electrophoresis of the substance to be electrophoresed in a second cavity.

Please replace the paragraph at page 23, line 24 - page 24, line 2 with the following amended paragraphs:

After first-dimensional electrophoresis, a connection medium is introduced to the intervening intermediate cavity. A buffer is used as the connection medium. The protein

comprised in the first dimensional electrophoretic medium can transfer through the buffer to the denatured gel for SDS-PAGE in the second cavity. SDS-PAGE is conducted at this stage. The individual proteins constituting the proteome developed in the first dimensional gel transfer to the nearest second dimensional gel retained in the second cavity, and are developed according to their molecular weight. Molecular weight markers may also be electrophoresed under the same conditions.

Please replace the paragraph at page 25, lines 19-20 with the following amended paragraph:

Fig. 3 is photographs showing how the intervening intermediate cavity prevents liquid transfer between the capillaries.

Please replace the paragraphs at page 26, line 3 - page 27, line 8 with the following amended paragraphs:

First, a number of second dimensional capillaries (second cavities) were placed to cross one first dimensional capillary (first cavity) at right angles. Next, two capillaries serving as intervening intermediate cavities were produced at the intersection of the first and second dimensional capillaries, parallel to the first dimensional capillary. The capillaries serving as intervening intermediate cavities were placed on both sides of the first dimensional capillary. All capillaries were formed by processing glass substrate materials with a dicing saw. More specifically, grooves of the size described below were formed on the surface of a 3 mm thick, 80 mm x 80 mm glass substrate material, and used as capillaries. The first dimensional capillary was placed perpendicular to the second dimensional capillaries, 1.8 mm from one end of the second dimensional capillaries. The distance between the first dimensional capillary and the capillaries serving as intervening intermediate cavities was 2.0 mm. The intervening intermediate cavities were placed on both sides of the first dimensional capillary.

First dimensional capillary: Width 1.1 mm x length 70 mm x depth 0.4 mm

Second dimensional capillary: Width 1.1 mm x length 60 mm x depth 0.3 mm

Capillary of the intervening intermediate cavity: Width 2.0 mm x length 50 mm x depth 0.5 mm

Fig. 1 shows the structure of the two-dimensional electrophoretic device produced, and Fig. 2 shows a photograph of the same. Such an arrangement enables control of

substance transfer to the second dimensional capillaries in either direction. The structure of the intervening intermediate cavity is not a complicated micromachine structure comprising valves or other mechanical components, but rather a groove which can be readily produced. When liquid has not been introduced, the intervening intermediate cavity prevents substance transfer from the intersection to the second dimensional cavity. Due to surface tension there is no movement of the substance to the intervening intermediate cavity at the intersection of the first cavity and the intervening intermediate cavity. Fig. 3 shows how the first and second dimensional capillaries are separated by an intervening intermediate cavity.

When moving the substance to be electrophoresed to a second dimensional cavity after completing the first dimensional isoelectric focusing, electroconductive aqueous solution is introduced to the two capillaries constituting the intervening intermediate cavity. The electroconductive aqueous solution functions as a connection medium. When the capillaries are filled with aqueous solution, the separation medium separating the solution of the first and second dimensions (air) is no longer present, and the solutions are thus in electrical contact. Under these conditions, applying voltage to the second cavity will start second dimensional electrophoresis. In other words, proteins separated in the first dimensional capillary will develop from the intersection nearest to the separation site of each protein, into the electrophoretic medium in the second cavity, via the intervening intermediate cavity.

Please replace the paragraph at page 27, lines 14-22 with the following amended paragraph:

The present invention provides methods for controlling the transfer of fluid substances, and/or substances comprised in fluid substances, by introducing a connection medium. By using the present invention, diffusion into the second dimensional electrophoretic medium can be suppressed during the first dimensional electrophoresis, even in two-dimensional electrophoresis. The methods of the present invention enable substance transfer to be controlled by the introduction of a connection medium to the intervening intermediate cavity. When a buffer is used as the connection medium, substance transfer can be realized by simply introducing a liquid to the intervening intermediate cavity. Accordingly, the present invention provides methods which readily and simply enable substance control.

Please replace the Abstract at page 32 with the following paragraph:

Transfer of fluid substances, and/or substances comprised in fluid substances, is controlled by introducing a separation medium, which prevents transfer of the fluid substances, and/or the substances comprised in the fluid substances, to an intervening intermediate cavity connecting a first cavity and a second cavity; and introducing a connection medium to replace the separation medium and thereby start substance transfer to the second cavity. Substance transfer may be readily controlled without relying on mechanical means. Based on the present invention, two-dimensional electrophoretic analysis can be readily implemented on a chip.